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Studies on the biology of the tree-frog *Hyla arborea* during the breeding season in North Western Italy (Amphibia, Anura, Hylidae)

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Some parameters on the breeding phenology and larval period of *Hyla arborea* during 1988 and 1989 spawning seasons were investigated in six ditches in North Western Italy.

Spawning period lasted from 31 to 36 days (from 15 April to 22 May) with two peaks of oviposition (one in April and one in May), and an interruption ranging from 5 to 10 days. Number of egg masses laid was positively correlated with water temperature.

The mean number of eggs estimated in 100 egg masses was 59 ± 23 SD. 93% of observed eggs were fertile. The percentage of unfertile eggs was significantly greater in larger masses.

Larval period ranged from the first days of May to the middle of July, with an average duration of 66-67 days.

INTRODUCTION

Although the reproductive biology of European Anuran Amphibians has been and continues to be object of numerous studies, only a few publications refer to the tree frog *Hyla arborea* (Linnaeus, 1758). This species shows a wide distribution in Europe (ARNOLD & BURTON, 1978), and in the recent past it was rather common. Now *H. arborea* is a vulnerable species in Northern Europe (STUMPEL & HANEKAMP, 1986) as well as in North Western Italy, where it shows a localized distribution (PAVIGNANO & GIACOMA, 1986).

H. arborea is a rather terrestrial species; it only lives in aquatic habitats during the reproductive period. The aquatic habitat consists of ponds in grasslands and marshes. Extent of aquatic vegetation cover, surrounding terrestrial habitat, level of human interference are discriminant parameters for this species in choosing breeding sites (PAVIGNANO et al., 1989 a). Alteration of these ecological parameters is probably the cause of current sporadic distribution of species. Where the habitat is suitable, *H. arborea* occurs usually with a numerous breeding population (PAVIGNANO et al., 1989 b); this is probably due to the several reproductions during the breeding season. The reproductive period of *Hyla* is in fact a long one (DIAZ-PANIAGUA, 1986; GARTON & BRANDON, 1975; PERRIL & DANIEL, 1983); in Northern Italy two spawn periods of *H. arborea* have been observed (PAVIGNANO, 1990).

There is no specific work on reproductive biology of *H. arborea*; in this study I investigated some parameters of the breeding phenology and larval period of *H. arborea* in six

ponds in North Western Italy during the years 1988 and 1989. Particular emphasis was given to gathering precise information concerning the duration of spawning period, the number of eggs per clump, the number of fertile eggs, and duration of metamorphosis of tadpoles.

Materials and methods

The study area has been described in detail in a former paper (Pagignano, 1989) and consists of a series of temporary draining ditches in fields, of which six sites were populated by *H. arborea*.

Observations were made every day during the breeding period.

For the estimation of the total spawn, all the egg masses at each pond were counted during every visit and the newly laid egg masses were marked on maps. The total number of eggs per clump was calculated by direct count from randomly selected egg masses and in each clump the number of fertile eggs was determined by stereo-microscope.

Tadpole populations in the ponds were sampled once every two days. The larvae, captured for each sample with a dipnet in different location in the ponds, were counted and larval stages (according to Gosner, 1960) of each species was determined. Tadpoles were then released. A small number of eggs and tadpoles was fixed in 60 % ethanol for microscopical observation of exact larval stage, and then photographed.

Maximum and minimum water and air temperature was recorded daily by a maximum/minimum thermometer placed in each pond, together with general conditions (i.e. if it had rained or not, etc.). The local climate is classified as continental with rainfall maxima in the middle of April (mean = 113mm) and November (mean = 79 mm); minima occurs in January (mean = 32 mm) and July (mean = 79 mm) (Durio et al., 1983).

Results

In Northern Italy, *H. arborea* begins to spawn between the middle and end of April. During the two years of my observations the spawning season lasted 36 days in 1988 (from 15 April to 20 May) and 31 days in 1989 (from 22 April to 22 May). In the 1988 breeding season a total of 404 laid egg masses (mean = 67.33 ± 22.06 SD for each pond) was recorded, and 354 (mean = 59.00 ± 27.63 SD) in 1989. No oviposition occurred when minimum water temperature was below 8°C and air temperature below 6°C. During the spawning period average water temperature varied between 10-22°C. Number of egg masses laid was positively correlated with average water temperature ($r = 0.469$, $p < 0.005$). In both years there were two peaks of oviposition (fig. 1) : 15 to 21 April and 15 to 18 May for 1988 (in those periods 82% of egg masses were laid, 60% of which in April) ; 25 to 29 April and 13 to 17 May for 1989 (84% of egg masses, 59% of which in April). The maximum spawning activity took place in April. An interruption of oviposition for about ten days in 1988 (5 to 14 May) and five days in 1989 (9 to 13 May) was observed. The laid egg masses were observed either before or after rainy days; no significant correlation was recorded between the number of laid egg masses and rain.

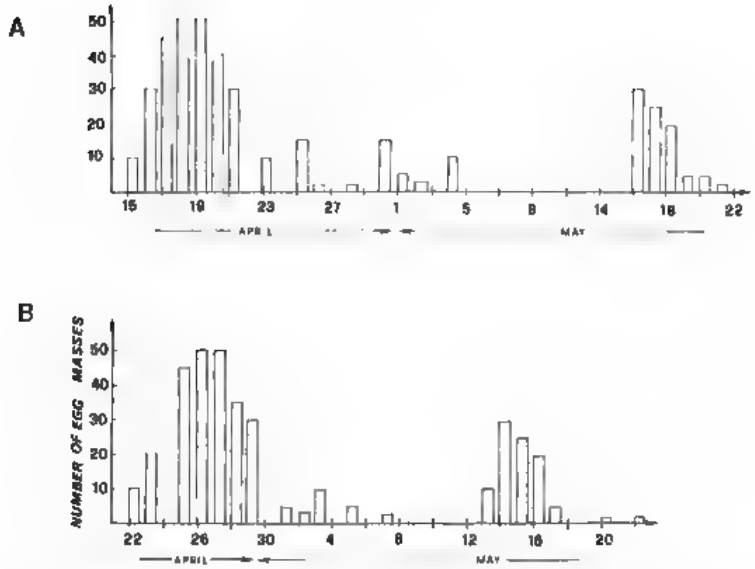

Fig. 1. – Number of egg masses per day laid in 1988 (a) and 1989 (b) spawning seasons.

The egg masses were laid in small groups, near the pond edges, at a depth of 5 to 10 cm, fixed on the vegetation. The shape of the egg masses was oval, the colour of the eggs was pale with grey animal hemisphere.

In the first days of October a further breeding activity was observed, revealing itself only by male calls. Males called from aquatic vegetation, but in a shorter and less frequent way than in April.

The number of eggs per mass determined by counting in 100 randomly selected egg masses is shown in Table I. The mean number of eggs per mass in the two years was 59 (± 23 SD) eggs. The difference in the mean number of eggs in the two years was not statistically significant (Student's $t = 1.75$, $p > 0.05$). 60% of egg masses included 60–100 eggs. Very small egg masses (20-30 eggs) were laid at the end of the spawning period and comprised 30% of total; there were also a few very large egg masses of more than 100 eggs comprising 5% of total. 93% of observed eggs were fertile; the percentage of unfertile eggs was significantly (Student's $t = 0.78$, $p < 0.05$) greater in large masses. In masses with 20-30 eggs, they were all fertile.

Table I. – Number of eggs per mass observed in 1988 and 1989 spawning seasons (*number of fertile eggs).

Year	N	Max	Min	M	SD
1988	50	125	20	57	28
		*120	20	54	27
1989	50	118	25	62	18
		*110	25	56	12
Total	100	125	20	59	23
		*120	20	55	20

Average larval period duration was of 67 days ± 1.02 SD in 1988 and 66 days ± 1.32 SD in 1989 (from the first days of May to the middle of July), from the first tadpoles leaving stage 25 (last embryonic stage according to GOSNER, 1960) until the last tadpoles completed their metamorphosis. Larval populations were composed of individuals at different development stages, because the various egg masses were laid in different times. There was a temporal overlap, lasting about 36 days, between tadpoles of different age and size classes. When the development of tadpoles from first ovipositions was between stage 34 and 40, there were tadpoles between stage 28 and 32; when the tadpoles from first ovipositions completed their metamorphosis, there were still tadpoles at stages 36 and 40.

DISCUSSION

The reproduction of Amphibians is usually related to weather factors, such as temperature and rainfall. *H. arborea* belongs to the type of Anurans which have a long spawning season; a first consequence of this is a relation with weather conditions. Although there were two oviposition peaks (about one month one after the other), this species seemed not to be particularly dependent on the rainfall, while no oviposition below 8°C water and 6°C air temperature was observed, and the observed oviposition was well correlated with temperature. Breeding activity not dependent on rain has also been observed in other species of *Hyla* (DIAZ-PANIAGUA, 1986).

A second consequence of the long spawning period was the temporal overlap of larval populations composed of individuals at different ages and sizes. The length of the larval period of *Hyla* is related to water temperature (DIAZ-PANIAGUA, 1986; STUMPEL & HANEKAMP, 1986); an increase of 5 – 8°C water temperature causes a shortening of about 10 days in *H. arborea*'s larval period (PAVIGNANO, 1990).

The variation in eggs number per mass shown by my results is in good agreement with LANZA (1983). The percentage of fertile eggs was related to the number of eggs per mass. Probably, as it happens in other Anuran species (HAAPENEN, 1982; SOFIANIDOU & KYRIAKOPOULOU-SKLAVOUNOU, 1983), the number of eggs laid by a female is dependent on its size. Quantitative information concerning the number and size of eggs from individual females, and relations with body size require further investigations.

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